In the Specification:

Please amend the specification as shown:

Please delete the paragraphs on page 2, line 4 to page 3, line 2 and replace it with the following paragraphs:

Metalloproteases are the most diverse of the four main types of protease, with more than 30 families identified to date. In these enzymes, a divalent cation, usually zinc, activates the water molecule. The zinc metalloproteases can be divided based on the zinc binding site into for example Zincins and Inverzincins (Hooper, N.M. 1994). The metal ion is held in place by amino acid ligands, usually three in number. The known metal ligands are His, Glu, Asp or Lys and at least one other residue which may play an electrophillic role is required for catalysis,. Of the known metalloproteases, around half contain an HEXXH **(SEQ ID NO: 89)** motif, which has been shown in crystallographic studies to form part of the metal-binding site.

A number of proteases dependent on divalent cations for their activity have been shown to belong to a single family, peptidase M16. Included are insulinase, mitochondrial processing protease, pitrilysin, nardilysin and a number of bacterial proteins. These proteins do not share many regions of sequence similarity; the most noticeable is in the N-terminal section. This region includes a conserved histidine followed, two residues later, by a glutamate and another histidine. In pitrilysin, it has been shown that this HXXEH **(SEQ ID NO: 1)** motif is involved in enzymatic activity (Becker et al. 1992); the two histidines bind zinc and the glutamate is necessary for catalytic activity. The X can be any amino acid. Non active members of this family have lost from one to three of these active site residues.

It has previously been suggested that one could provide host cells and methods of producing proteins by expressing significantly reduced levels of a genetical modification in order to express significantly reduced levels of a metalloprotease containing an HEXXH **(SEQ ID NO: 89)** motif in a filamentous fungal host cell, in e.g. US 5,861,280 (WO 98/12300).

Others have provided a protease deficient filamentous fungus which is characterised in that the filamentous fungus contains a site selected disruption of DNA that results in the filamentous fungus having reduced metalloprotease activity and isolated DNA sequences encoding a protein having metalloprotease activity, which is obtainable from a filamentous fungus (WO 97/46689). Again this metalloprotease contains an HEXXH **(SEQ ID NO: 89)** motif.

However, metalloproteases which can be reduced by a genetical modification in order to express significantly reduced levels of said metalloprotease in a non-filamentous fungal host cell and other cells containing an motif other than HEXXH **(SEQ ID NO: 89)** have never been described.

Please delete the paragraph on page 9, lines 14-17 to and replace it with the following paragraph:

Figure 1. Cholecystokinin expression construct. PreproMfα1p-proCCK fusion protein **(SEQ ID NOS 53-55, respectively, in order of appearance)** with the amino acid sequences around the fusion site and of the primary cleavage sites shown. The major forms of secreted CCK with their N- and C-terminal amino acid residues are shown below.

Please delete the paragraphs on page 11, line 31 to page 12, line 13 and replace it with the following paragraphs:

Figure 12. Secreted proCCK fragments identified by mass spectrometry **(SEQ ID NOS 43-52, respectively, in order of appearance)**. The CCK-numbers refer to C-terminal amidated CCK. The molecular masses are given as monoisotopic values except for * which denote average value. Strain A, vacuolar protease-deficient strain (BJ2168), and B, the isogenic strain with *KEX1 KEX2* disruptions (LJY22).

Figure 13. Model for the production of the C-terminally extended CCK (A) **(SEQ ID NOS 53 & 54, respectively, in order of appearance)** and GLP2 (B) **(SEQ ID NOS 53 & 69 respectively, in order of appearance)**. Expression of these fusion peptides should be performed in a *sec61* mutant, or the pre-sequence of the α-mating factor should be

removed to avoid translocation into the ER. The amino acid sequences around the fusion sites are shown. Underlined are the N- and C-terminal amino acids of the Gly-extended CCK-22 and GLP1.

Figure 14

A. The preproMfα1p proBNP expression construct **(SEQ ID NOS 70 & 71)**.

B. The preproMfα1p KREAEA-BNP-32 expression construct **(SEQ ID NOS 72 & 73)**.

C. The preproMfα1p KR-BNP-32 expression construct **(SEQ ID NOS 74 & 75)**.

Please delete the paragraph on page 14, lines 5-11 and replace it with the following paragraph:

The metalloproteases which are to be down regulated according to the present invention do not share many regions of sequence similarity; the most noticeable is in the N-terminal section. This region includes a conserved histidine followed two residues later by a glutamate and another histidine. In pitrilysin, it has been shown that this HXXEH **(SEQ ID NO: 1)** motif is involved in enzymatic activity; the two histidines bind zinc and the glutamate is necessary for catalytic activity. Non active members of this family have lost from one to three of these active site residues.

Please delete the paragraphs on page 14, line 27 to page 15, line 15 and replace it with the following paragraphs:

Among such sequence similarities several individual amino acids are highly conserved and are easily recognisable in specific positions navigated from the HXXEH **(SEQ ID NO: 1)** motif.

Thus, one embodiment of the present invention relates to a host cell, wherein the metalloprotease comprises a glutamic acid residue between 70 and 80 amino acids C-terminal of the second His residue in the HXXEH **(SEQ ID NO: 1)** motif.

A further embodiment of the present invention relates to a host cell, wherein the metalloprotease comprises a glysine residue 3 amino acids N-terminal of the first His residue in the HXXEH **(SEQ ID NO: 1)** motif.

Another embodiment of the present invention relates to a host cell, wherein the metalloprotease comprises a glysine residue 5 amino acids C-terminal of the second His residue in the HXXEH **(SEQ ID NO: 1)** motif.

One further embodiment of the present invention relates to a host cell wherein the metalloprotease comprises a lysine residue 8 amino acids C-terminal of the second His residue in the HXXEH **(SEQ ID NO: 1)** motif.

Also, one embodiment of the present invention relates to a host cell, wherein the metalloprotease comprises a tyrosine residue 9 amino acids C-terminal of the second His residue in the HXXEH **(SEQ ID NO: 1)** motif.

Furthermore, the present invention relates to a host cell, wherein the metalloprotease comprises a proline residue 10 amino acids C-terminal of the second His residue in the HXXEH **(SEQ ID NO: 1)** motif.

Please delete the paragraph on page 15, lines 25-27 and replace it with the following paragraph:

In a presently most preferred embodiment, the invention relates to a host cell, wherein the metalloprotease comprises a NAXTXXXXT **(SEQ ID NO: 76)** motif between 20 and 30 amino acids C-terminal of the second His residue in the HXXEH **(SEQ ID NO: 1)** motif.

Please delete the paragraph on page 28, lines 16-18 and replace it with the following paragraph:

Thus, it will be understood that any feature and/or aspect discussed above in connection with any of these different family annotations apply by analogy to the metalloprotease described herein, which all include the HXXEH **(SEQ ID NO: 1)** motif.

Please delete the paragraph on page 39, lines 7-14 and replace it with the following paragraph:

Messenger RNA was isolated from a 500 mg Biopsy from human heart using the Quickprep Micro mRNA purification Kit (Amersham Pharmacia Biotech). First strand cDNA was prepared from 2 µg mRNA in a reaction containing, 2.5 µl 10x first strand buffer (Promega), 2.5 µl 100 mM DTT, 2.5 µl 10 mM dNTP, 2.5 µl Na pyrophosphate, 10 pmol Oligo(dT)18 **(SEQ ID NO: 77)**, 10 units reverse transcriptase, AMV (Promega), and H2O to 25 µl. Messenger RNA and Oligo(dT)18 **(SEQ ID NO: 77)** was heated to 70°C for 5 min cooled on ice for 5 min prior to cDNA synthesis. The first strand cDNA syntesis was performed at 42°C for 60 min.

Please delete the paragraph on page 39, line 35 to page 40, line 5 and replace it with the following paragraph:

Furthermore, two additional constructs have been made, in which the proBNP fragment (1-76) has been removed. These constructs are similar to do the preproMfα1p proBNP, but do only synthesise BNP-32. In the first construct, the Kex2p cleavage site and the spacer peptide of the preproMfα1p has been sustained (KREAEA **(SEQ ID NO: 78)**)(Figure 14B), whereas in the other construct, the spacer peptide has been removed (Figure 14C). Analysis of the BNP-32 expression from wild type yeast and the isogenic CYM1 disruptant will be analysed by RIA's using the Shionoria-BNP system from Electra-Box Diagnostica ApS. This assay is specific for BNP-32.

Please delete the paragraph on page 41, lines 9-34 and replace it with the following paragraph:

By alignment of the orthologous Cym1 proteins of Saccharomyces kluveri and Schizosaccharomyces pombe to Cym1p from Saccharomyces cerevisiae, there was identified a number of identical amino acid sequences. From these sequences it is possible to syntesize degenerated oligonucleotides (Table 3) that will bind to the complementary DNA strands of CYM1 in all three species, and thus to the CYM1 gene of Pichia. Amplification of the genomic sequence will initially be carried out by using high quality genomic DNA as template, Pichia-CYM1-Ia and Pichia-CYM1-Ib and the Pwo polymerase (Roche). The amplified sequence with an expected size of approximately 525 bp will be cloned in pBlunt or a similar vector and sequenced with vector specific primers. If no band appear from the initial amplification, a second round of PCR will be performed with the two nested primers, Pichia-CYM1-IIa and Pichia-CYM1-IIb using 1 µl of the first PCR reaction as template. The expected product is approximately 270 bp and will be cloned in pBlunt and sequenced with M13 foreward and M13 reverse primers. From the obtained sequence there will be synthesized sequence specific primers, two nested sense and two nested antisense specific primers. Using one of the sense primers it is possible to obtain a PCR product with Pichia-CYM1-IIIb using high quality genomic DNA as template. This product of ~2100 bp will be cloned and sequenced. If it fails to produce a band of ~2100 bp, it would be nessessary to isolate mRNA from Pichia, produce double stranded cDNA and ligate adaptors to the ends as described by the manual to the Clontech Marathon cDNA Amplification Kit (BD (Becton, Dickinson and Company)). Using the two sequence specific sense primers it is possible to obtain the 3' end of the mRNA of approximately 2600 bp and the sequence specific antisense primers to amplify the 5' end including the sequence encoding the hypothetical active site, HXXEH **(SEQ ID NO: 1)**motif. Synthesis of sequence specific oligonuleotides from the 5' and 3' untranslated region, full-length cDNA encoding the Cym1 orthologue in Pichia can be cloned.

Please delete Table 3 on page 41 and replace it with the following table:

Table 3

Amino acid sequence **(SEQ ID NO: 79)**	K Y P V R D P
Oligo Pichia-CYM1-Ia **(SEQ ID NO: 80)**	5' AARTAYCCXGTXMGXGAYCC 3'
Amino acid sequence **(SEQ ID NO: 81)**	H P S N A K
Oligo Pichia-CYM1-Ib **(SEQ ID NO: 82)**	3' GTRGGXWSXTTRCGXTTY 5'
Amino acid sequence **(SEQ ID NO: 83)**	D P F F K M
Oligo Pichia-CYM1-IIa **(SEQ ID NO: 84)**	5' GAYCCXTTYTTYAARATG 3'
Amino acid sequence **(SEQ ID NO: 85)**	G V V Y N E M
Oligo Pichia-CYM1-IIb **(SEQ ID NO: 86)**	3' CCXCAXCAXATRTTRCTYTAC 5'
Amino acid sequence **(SEQ ID NO: 87)**	E K G G A Y G
Oligo Pichia-CYM1-IIIb **(SEQ ID NO: 88)**	3' CTYTTYCCXCCXCGXATRCC 5'

X-Inosine, degenerated oligonucleotides follow the International Union of Biochemistry (http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html).

Please delete the paragraph on page 51, line 35 to page 52, line 4 and replace it with the following paragraph:

The propeptide or prepropeptide of interest will then be cytosolicly located and a potential substrate for Cym1p. Release of the peptide from its precursor will be carried out by the Cym1p activity by introduction of the cleavage site seen from proCCK, which results in the release of Gly-extended CCK-22 after endoproteolytic processing C-terminal to Lys61 (Ser-Ile-Val-Lys^{61} ↓ **(Residues 3-6 of SEQ ID NO: 54)**) (Fig. 13A). If the peptide of interest is GLP1, synthesis can be performed as a fusion to a Cym1p cleavage site, which could be part of proCCK (Fig. 13B). The peptide of interest will then accumulate in the cytosol and can be purified from sedimented cells after lysis.